BET inhibitor JQ1 induces apoptosis of ovarian and endometrial endometrioid carcinoma cells by downregulating *c-Myc*

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Abstract. Although ovarian endometrioid carcinoma (OEC), frequently associated with endometrial endometrioid carcinoma (EEC), is often diagnosed at an early stage, the prognosis remains poor. The development of new, effective drugs to target these cancers is highly desirable. The bromodomain and extra-terminal domain (BET) family proteins serve a role in regulating transcription by recognizing histone acetylation, which is implicated in several types of cancer. BET inhibitors have been reported as promising cancer drugs. The present study aimed to assess the role of JQ1, a BET inhibitor, in ovarian and endometrial cancers. The sensitivity of OEC and EEC cell lines to JQ1 was assessed using cell viability and colony formation assays. Additionally, western blotting and cell cycle assays were performed to evaluate changes in c-Myc expression and apoptosis markers. Cell proliferation and colony formation assays revealed significant tumor suppression in both OEC and EEC cell lines in response to JQ1 treatment. Furthermore, treatment with JQ1 induced a decrease in *c-Myc* expression and an increase in apoptosis markers, such as cleaved PARP and the cell population in the sub-G1 phase, in both OEC and EEC cell lines. The findings of the present study indicate that JQ1 may induce cell death through c-Myc inhibition and could be a potentially novel therapeutic agent in the treatment in OEC and EEC. However, the direct mechanism, has not been fully elucidated, warranting further investigation.

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Abbreviations: BET, bromodomain and extra-terminal domain; BRD, bromodomain; DMSO, dimethyl sulfoxide; EEC, endometrial endometrioid carcinoma; FBS, fetal bovine serum; OEC, ovarian endometrioid carcinoma; PARP, poly ADP ribose polymerase; PBS, phosphate-buffered saline; PVDF, polyvinylidene difluoride; SDS, sodium dodecyl sulfate; SEOC, simultaneous endometrial and ovarian cancer

Key words: apoptosis, bromodomain and extra-terminal domain protein, epigenetics, c-Myc, ovarian endometrioid carcinoma, endometrioid endometrial carcinoma

Introduction

Ovarian cancer is the deadliest gynecological cancer, as it is often detected only at an advanced stage and shows frequent recurrence (1). The main histological types of epithelial ovarian cancer are serous, clear cell, endometrioid and mucinous carcinomas. Among these, endometrioid carcinomas account for 10-15% of all epithelial ovarian cancers (2) and are classified as grade 1, 2 or 3. Although >70% of endometrioid carcinomas are diagnosed at Stage I or II, the prognosis of patients with this type of cancer remains poor (3). In Japan and other Asian countries, the incidence rates of endometrioid and clear cell carcinomas are higher than in other regions (4). The risk factors include endometriosis, Lynch syndrome and intestinal dysbiosis (5-7). Ovarian endometrioid carcinoma (OEC) is often associated with endometrial cancer, which is also called 'simultaneous endometrial and ovarian cancer' (SEOC) (8). In such cases, distinguishing whether the cancer is an individual tumor or a metastatic case is difficult, and controversy regarding this exists currently (8).

In terms of epigenetics, the main factors affecting gene expression regulation are DNA methylation and histone modifications (9). Among histone modifications, histone acetylation, such as H3K27Ac, promotes transcriptional activity by transforming the chromatin state into an open state (10). This process is orchestrated by three factors: i) Histone acetyltransferases, which serve the role of 'writer'; ii) bromodomain (BRD) proteins, which are 'reader'; and iii) histone

deacetylation, which function as 'eraser' (11-13). Therefore, BRD proteins, the members of the bromodomain and extra-terminal domain (BET) family (14), contribute to transcriptional regulation by recognizing histone acetylation and recruiting chromatin- and transcription-related factors (13). In particular, BRD4 promotes transcription initiation by binding to the acetyl group of lysine in histones H3 and H4 via its own bromodomain (15). BET proteins, particularly BRD4, have been implicated in human diseases, particularly cancer (16). *c-Myc*, which is often upregulated in cancer, is the main downstream gene regulated by BRD4 (17). BRD4 inhibition has been reported to downregulate *c-Myc* expression in several tumor types. Accordingly, BET inhibitors, including JQ1, have been reported as novel therapeutic agents in the treatment of several cancers (18). However, no studies have reported on the effects of JQ1 in OEC, to the best of our knowledge. Therefore, the present study aimed to assess the antitumor effect of JQ1 in OEC and endometrial endometrioid carcinoma (EEC) to develop a novel treatment for SEOC.

Materials and methods

Cell lines. A total of three OEC cell lines (A2780, TOV112D and OVK18) and three EEC cell lines (HEC265, human endometrioid adenocarcinoma G1; HEC151, human endometrioid adenocarcinoma G2; and HEC50B, human endometrioid adenocarcinoma G3) we used in the present study.

A2780 cells (European Collection of Authenticated Cell Cultures) were cultured in RPMI-1640 medium (FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.). TOV112D cells (American Type Culture Collection) were cultured in MCDB 105 medium (Sigma-Aldrich; Merck KGaA) supplemented with 15% heat-inactivated FBS. OVK18 cells (RIKEN BioResource Center) were cultured in minimum essential medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% heat-inactivated FBS.

HEC265, HEC151 and HEC50B cell lines (JCRB Cell Bank) were cultured in Eagle's minimum essential medium (FUJIFILM Wako Pure Chemical Corporation) containing 10% heat-inactivated FBS. All cell lines were maintained at 37˚C in a humidified atmosphere with 5% CO_2. The mutation status of OEC and EEC cell lines was searched using the Cancer Cell Line Encyclopedia data (https://sites.broadinstitute.org/ccle/).

Small interfering (si)RNA transfection. A2780 and HEC50B cells were transfected with 10 nM siRNAs at 37˚C for 3.5 h using Lipofectamine™ RNAiMAX Transfection Reagent (cat. no. 13778150; Invitrogen™; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. A total of 72 h after siRNA transfection, RNA extraction, protein extraction and cell viability assay were performed. siBRD4 #1 (sense: 5'-GUGCUGAUGUCCGAUUGAU-3' and antisense: 5'-AUCAAUCGGACAUCAGCAC-3'), siBRD4 #2 (cat. no. NM_058243, SASI_Hs01_00126965) and a negative control siRNA (siNC; MISSION® siRNA Universal Negative Control; cat. no. SIC001) were purchased from Sigma-Aldrich (Merck KGaA).

RNA extraction and reverse transcription(RT)-quantitative (q)PCR. After siRNA transfection, total RNA from A2780 and HEC50B cells was extracted using RNeasy® Mini Kit (cat. no. 74104; Qiagen, Inc.). cDNA synthesis from mRNA was performed using ReverTra Ace™ qPCR Master Mix with gDNA Remover (cat. no. FSQ-301; Toyobo Co., Ltd.) with the following steps: 37˚C for 15 min, 50˚C for 5 min and 98˚C for 5 s. The mRNA expression levels were measured using qPCR using the One-Step SYBR Prime Script RT-PCR Kit (cat. no. RR064A; Takara Bio, Inc.) and the QuantStudio™ 1 Real-Time PCR System (Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: Initial denaturation step at 98˚C for 2 min, followed by 40 cycles at 98˚C for 10 s, 60˚C for 10 s and 72˚C for 30 s. The mRNA expression levels were normalized to the mRNA levels of β-actin. The relative mRNA expression level was calculated using the $2^{-\Delta\Delta Cq}$ method (19). The sequences of primers were as follows: β-actin, (forward) 5'-CACACTGTGCCCATCTACGA-3' and (reverse) 5'-CTCCTTAATGTCACGCACGA-3'; and BRD4, (forward) 5'-GTGGTGCACATCATCCAGTC-3' and (reverse) 5'-CCGACTCTGAGGACGAGAAG-3'.

Cell viability assay. The OEC cell lines (A2780, TOV112D and OVK18; $1x10^4$ cells/well) and EEC cell lines (HEC265, HEC151 and HEC50B; $4x10^3$ cells/well) were seeded on 48-well plate and treated with JQ1 (cat. no. HY-13030; MedChemExpress) for 72 h. After treatment, cells were incubated in a 10% Cell Count Kit-8 solution (Dojindo Laboratories, Inc.) for 2 h. The optical density at 450 nm was measured using a microplate reader (BioTek; Agilent Technologies, Inc.). Cell viability was normalized using 0.1% dimethyl sulfoxide (DMSO; Sigma-Aldrich) as the control.

Colony formation assay. The OEC cell lines (A2780, TOV112D and OVK18) and EEC cell lines (HEC265, HEC151 and HEC50B) were seeded at a density of $2x10^3$ cells/well on 6-well plates. After overnight incubation at 37˚C, the cells were treated with JQ1 (0.1, 0.2 and 0.5 μM) or 0.1% DMSO for 10 days to assess colony formation. The medium was replaced every 3-4 days. The plates were then washed with phosphate-buffered saline (PBS). Colonies were fixed with 100% methanol at room temperature (RT) for 2 h and stained with Giemsa stain (FUJIFILM Wako Pure Chemical Corporation) at RT for 1 h. The colonies (>50 cells) were counted manually under a microscope and normalized to the number of colonies treated with 0.1% DMSO.

Protein extraction and western blotting. The OEC cell lines (A2780, TOV112D and OVK18; $1x10^5$ cells/dish) and EEC cell lines (HEC265, HEC151 and HEC50B; $4x10^4$ cells/dish) were plated onto a 6-cm dish and treated with JQ1 (1 μM) or 0.1% DMSO at 37˚C for 72 h. Protein was extracted using a lysis buffer [0.1 M Tris-HCl; pH 7.5; 10% glycerol and 1% sodium dodecyl sulfate (SDS)]. The extracted proteins were boiled for 5 min and centrifuged at 4˚C for 10 min at 20,000 x g. The protein concentration was measured using a BCA protein assay kit (cat. no. 06385-00; Nacalai Tesque, Inc.). Each sample (15 μg/lane) was separated using SDS-PAGE (Mini-PROTEAN® TGX™ Precast Protein Gels (Any kD™); Bio-Rad Laboratories, Inc.) and transferred to polyvinylidene

difluoride (PVDF) membranes using Trans-Blot Turbo Mini PVDF transfer packs (Bio-Rad Laboratories, Inc.). Blocking was performed with 5% skim milk at RT for 1 h. The membrane was incubated with the primary antibodies at 4˚C overnight and incubated with the secondary antibodies at RT for 1 h. Protein expression was measured using the Amersham™ ECL™ Select (Cytiva), and the emitted signals were imaged using the ImageQuant™ LAS 4000 system (Cytiva). The following primary antibodies were used for immunoblotting: Rabbit anti-BRD4 (1:1,000; cat. no. 13440; Cell Signaling Technology, Inc.), rabbit anti-cleaved poly ADP ribose polymerase (PARP; 1:1,000; cat. no. 5625; Cell Signaling Technology, Inc.), rabbit anti-c-Myc (D84C12; 1:1,000; cat. no. 5605; Cell Signaling Technology, Inc.), PARP (1:1,000; cat/ no. 9542; Cell Signaling Technology, Inc.) and mouse anti-β-actin (1:7,000; cat. no. A2228; Sigma-Aldrich; Merck KGaA). For all primary antibodies, the incubation condition was at 4˚C overnight. The secondary antibodies used were as follows: Anti-mouse IgG HRP-linked (1:5,000; cat. no. 7076; Cell Signaling Technology, Inc.) and anti-rabbit IgG HRP-linked (1:3,000; cat. no. 7074; Cell Signaling Technology, Inc.). The semi-quantified values of the target protein were normalized by dividing them by the semi-quantified values of each β-actin in the same sample. Subsequently, using the value of DMSO as 1, the values of JQ1 were normalized. The values were semi-quantified using ImageJ 1.53 (National Institutes of Health). Graphs were subsequently constructed using the normalized values obtained from three independent experiments.

Cell cycle assay. The OEC cell lines (A2780, TOV112D and OVK18; $2x10^6$ cells/dish) and EEC cell lines (HEC265, HEC151 and HEC50B; $1x10^6$ cells/dish) were plated on a 10-cm dish with JQ1 (5 μM) or 0.1% DMSO and incubated at 37˚C for 96 h. Subsequently, the cells were harvested with trypsin, washed with PBS and fixed in 70% ethanol at -20˚C overnight. After washing twice with PBS, the samples were stained with propidium iodide (PI; Sigma-Aldrich; Merck KGaA) at 4˚C for 15 min. Cell cycle analysis was performed using flow cytometry with a BD FACSCalibur HG Flow Cytometer (BD Biosciences) and Cell Quest Pro software v. 6.1 (BD Biosciences). Data were assessed using FlowJo software, version 10 (BD Biosciences).

Kaplan-Meier survival analysis. The overall survival (OS) was analyzed using the Kaplan-Meier method with TCGA datasets in cBioPortal for Cancer Genomics (www.cbioportal.org). The dataset TCGA-OV (20) for ovarian cancer and PanCanAtlas (21) for endometrioid cancer were analyzed. The cases were categorized into high and low groups, based on BRD4 expression levels. Statistical significance was determined using the log-rank test.

Statistical analysis. Data are presented as the mean ± standard deviation of >3 independent experiments. Data were analyzed using Microsoft Excel 2016 (Microsoft Corporation) and GraphPad Prism 10 (Dotmatics). For comparisons between two groups, the unpaired Student's t-test was used. For comparisons among ≥3 groups, one-way analysis of variance followed by Dunnett's post hoc test was used. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Expression of BRD4 is associated with prognosis in patients with ovarian and endometrial carcinoma. Taking into account previous reports suggesting an association between the expression levels of BRD4 and prognosis in other cancer types (22-24), the present study assessed the association between BRD4 expression and prognosis in patients with epithelial ovarian carcinoma and endometrioid endometrial carcinoma using RNA-seq data from TCGA. OS was significantly worse in the BRD4-High than in the BRD4-Low group for both ovarian carcinoma (P=0.016; Fig. S1A) and endometrial carcinoma (P=0.033; Fig. S1B).

JQ1 suppresses cell proliferation in OEC and EEC cell lines. To evaluate the antitumor effect of JQ1 on OEC and EEC, the present study performed cell viability assays on three OEC and three EEC cell lines treated with 0.01-50 μM JQ1 for 72 h. Cell viability decreased in a dose-dependent manner in response to JQ1 treatment (Fig. 1). The IC_{50}, half maximal inhibitory concentration (IC_{50}) of JQ1 for A2780, TOV112D, OVK18, HEC265, HEC151 and HEC50B cells was 0.41, 0.75, 10.36, 2.72, 0.28 and 2.51 μM, respectively.

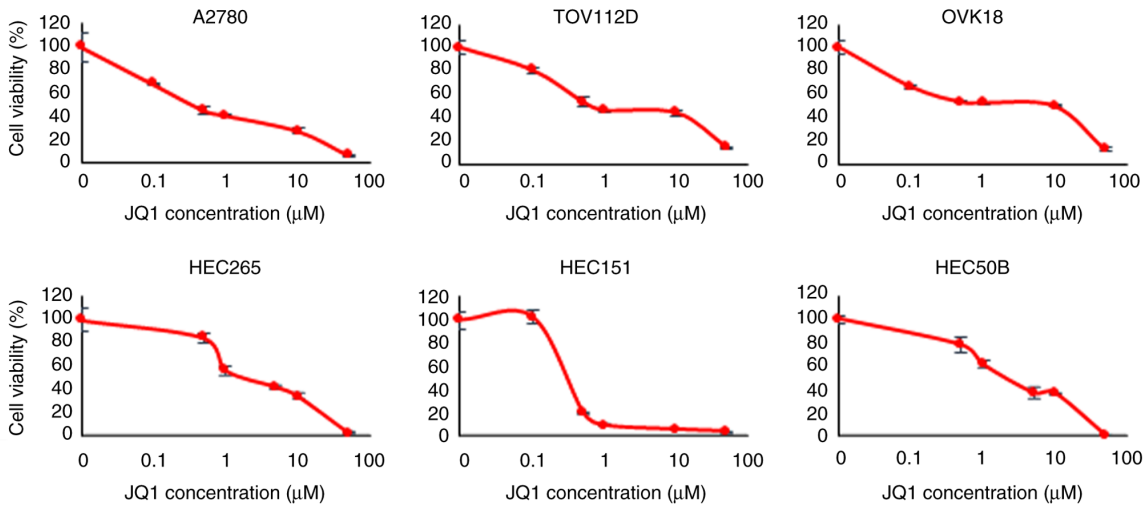
Furthermore, the common mutation status for each cell line is presented in Table SI. No mutation status indicative of JQ1 sensitivity was observed.

Colony formation assays were also performed to evaluate the long-term cytotoxicity of JQ1. Colony formation was significantly suppressed in a dose-dependent manner in all cell lines, in comparison with the control (Figs. 2 and 3). A low dose of JQ1 (0.1 μM) was sufficient to affect the long-term clonogenicity of OEC and EEC cell lines. The number of colonies in each treatment group is presented in Table SII.

JQ1 suppresses c-Myc expression. Western blot analyses were performed to assess the effect of JQ1 treatment on *c-Myc* expression in OEC and EEC cells. The *c-Myc* expression significantly decreased in all six cell lines after treatment with 1 μM JQ1 for 72 h, in comparison with the control (Fig. 4).

JQ1 induces apoptosis. Immunoblotting and cell cycle assays were performed to determine whether the decrease in cell viability was associated with apoptosis. As indicated by the western blotting results (Fig. 4), the expression levels of cleaved PARP (an apoptosis marker) were increased in A2780, TOV112D, HEC265, HEC151 and HEC50B cells treated with 1 μM JQ1 for 72 h. However, in OVK18 cells, for which a relatively high JQ1 IC50 value was obtained, the cleaved PARP level was not notably elevated with 1 μM, but was markedly elevated with 5 and 10-μM JQ1 treatment (Figs. 4A and S2). Furthermore, to demonstrate the increase in cleaved PARP, the present study confirmed that the expression level of the PARP protein itself did not increase (Figs. 4A, C and S2). The same processed protein samples were used for this experiment.

Moreover, the cell cycle analysis results revealed that the 5-μM JQ1 treatment significantly increased the population of cells in the sub-G1 phase in all six cell lines (Fig. 5). Consistent with the expression of cleaved PARP (Fig. 4), the



Cell line	IC_{50} (μM)
A2780	0.41
TOV112D	0.75
OVK18	10.36

Cell line	IC_{50} (μM)
HEC265	2.72
HEC151	0.28
HEC50B	2.51

Figure 1. JQ1 suppresses cell proliferation in ovarian endometrioid carcinoma and endometrial endometrioid carcinoma cell lines. Each cell line was treated with JQ1 at a concentration of 0.01-50 μM for 72 h. Cell viability was normalized to that of 0.1% DMSO. The IC_{50} value of JQ1 ranged from 0.28-10.36 μM. IC_{50}, half maximal inhibitory concentration.

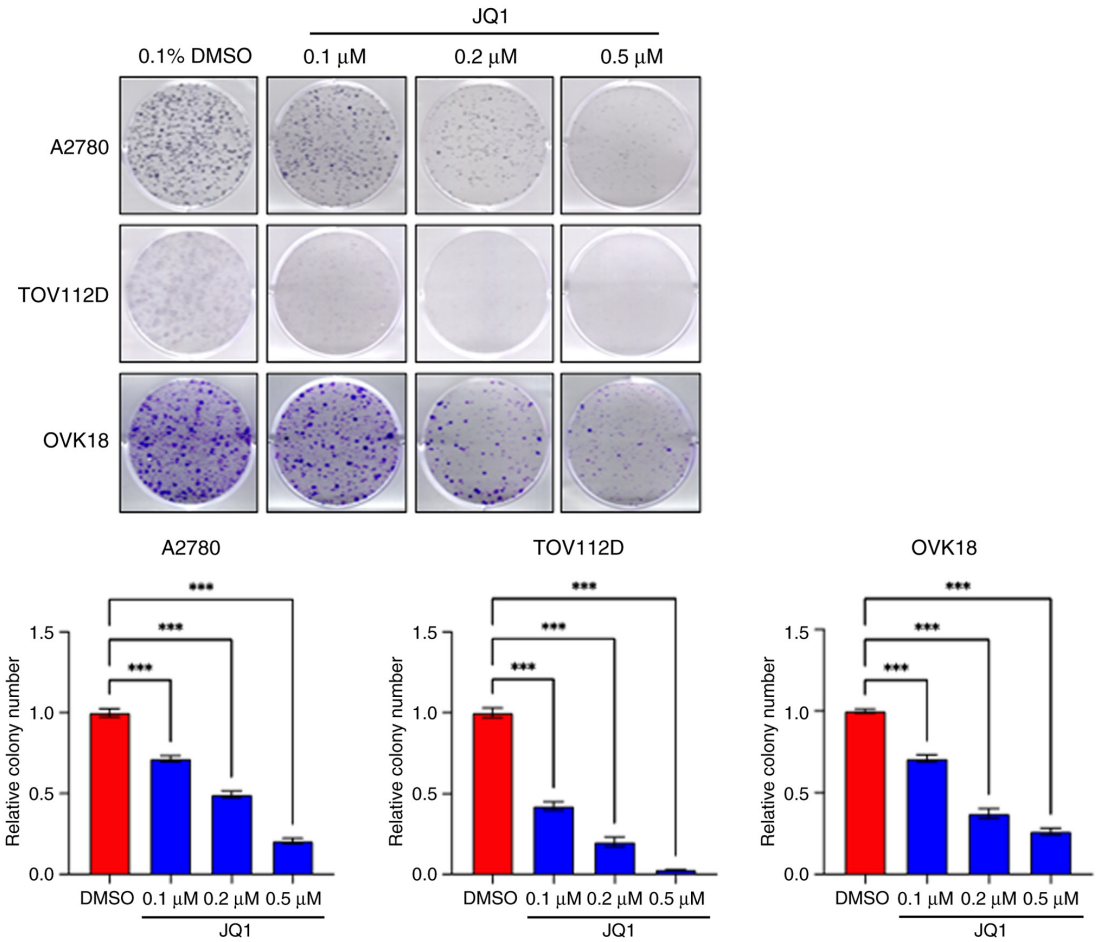


Figure 2. Colony formation assay of ovarian endometrioid carcinoma cell lines treated with JQ1. Cells were treated with JQ1 (0.1, 0.2 and 0.5 μM) or 0.1% DMSO for 10 days. Colony formation of all cell lines was significantly suppressed in a dose-dependent manner. ***P<0.001 vs. 0.1% DMSO.

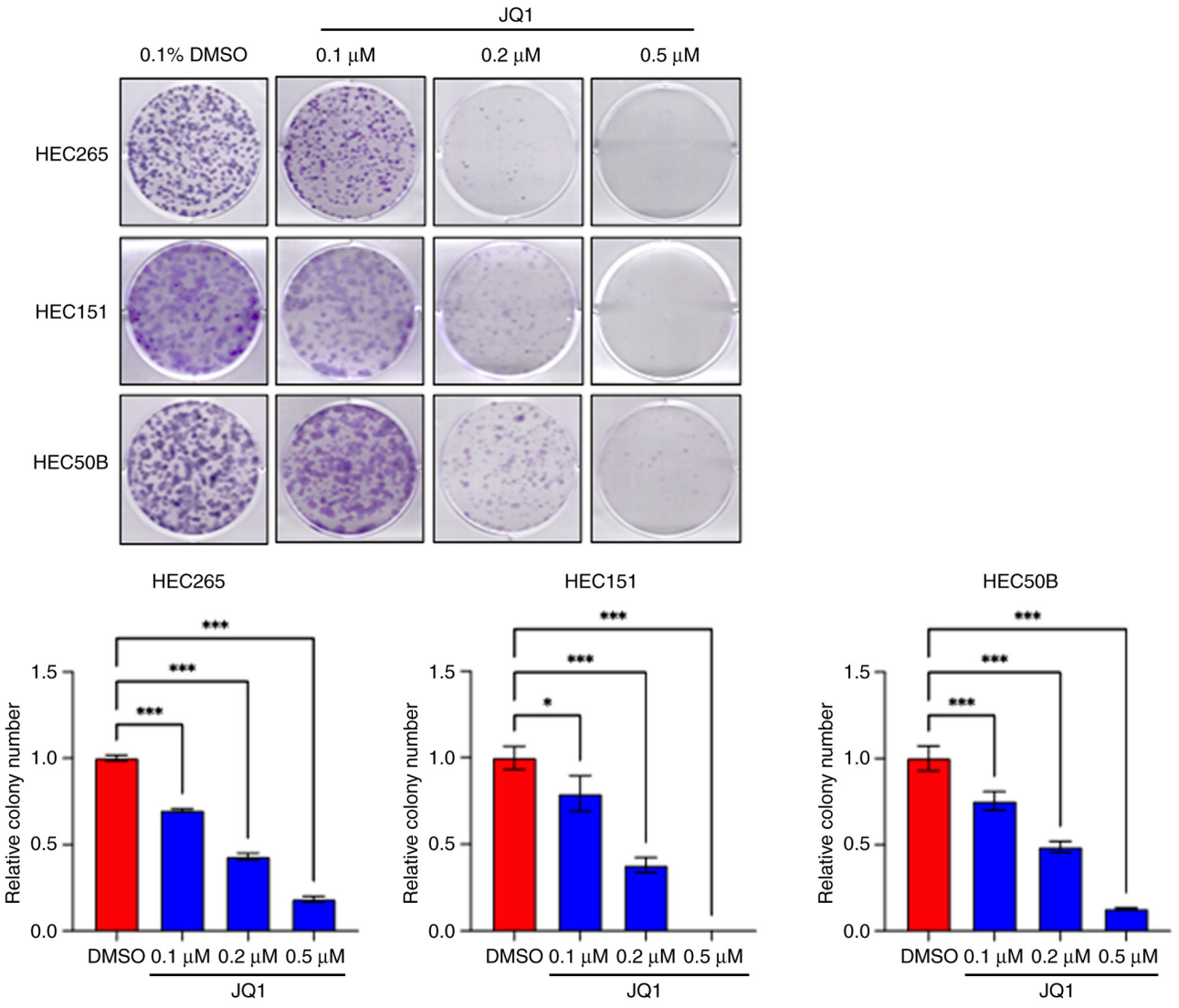


Figure 3. Colony formation assay of endometrial endometrioid carcinoma cell lines treated with JQ1. Cells were treated with JQ1 (0.1, 0.2 and 0.5 μM) or 0.1% DMSO for 10 days. Colony formation of all cell lines was significantly suppressed in a dose-dependent manner. *P<0.05; ***P<0.001, vs. 0.1% DMSO.

cell population was lowest in OVK18 cells among all the cell lines (Fig. 5). Collectively, these results indicate that JQ1 induced apoptosis and suppressed the proliferation of OEC and EEC cells.

BRD4 knockdown suppresses cell proliferation and induces apoptosis. The effect of BRD4 knockdown on cell proliferation was also evaluated. BRD4 knockdown tended to suppress cell proliferation in both OEC and EEC cell lines, compared with the negative control (Fig. S3A and B). Similar to the inhibitor experiment findings, BRD4 knockdown was also associated with a marked decrease in c-Myc and an increase in cleaved PARP expression levels (Fig. S3C).

Discussion

c-Myc is involved in several functions such as cell proliferation, cell immortalization and promotion of metastasis. Most of these functions reflect the function of c-Myc as a transcription factor. Furthermore, previous studies using chromatin immunoprecipitation experiments with BRD4 protein reported that *c-Myc* gene transcription is regulated by BET. Transcription of the *c-Myc* gene was also repressed following treatment with the BET inhibitor JQ1 (23,25).

Histone acetylation is a crucial epigenetic regulatory mechanism. The BET family and histone deacetylase (HDAC) family both regulate the expression of important cancer genes and tumor suppressor genes (26,27). HDAC inhibitors are clinically used as anticancer agents, and synergistic effects of combining BET inhibitors with HDAC inhibitors have been reported (28). BET inhibitors are currently undergoing clinical trials and are anticipated as promising new cancer therapeutics.

Although many studies on BET inhibitors for ovarian cancer have been published (29-32), the present study is the first in which JQ1 has been assessed in an OEC context, to the best of our knowledge. OEC is one of the four main histological types of epithelial ovarian cancer, with a high incidence in Japan and other Asian countries (4). Although OEC is often diagnosed at an early stage (stage I or II), the prognosis of certain patients remains poor (33). Endometrioid and clear cell carcinoma of the ovary have often been associated with endometriosis (5), and patients with endometriosis are at a

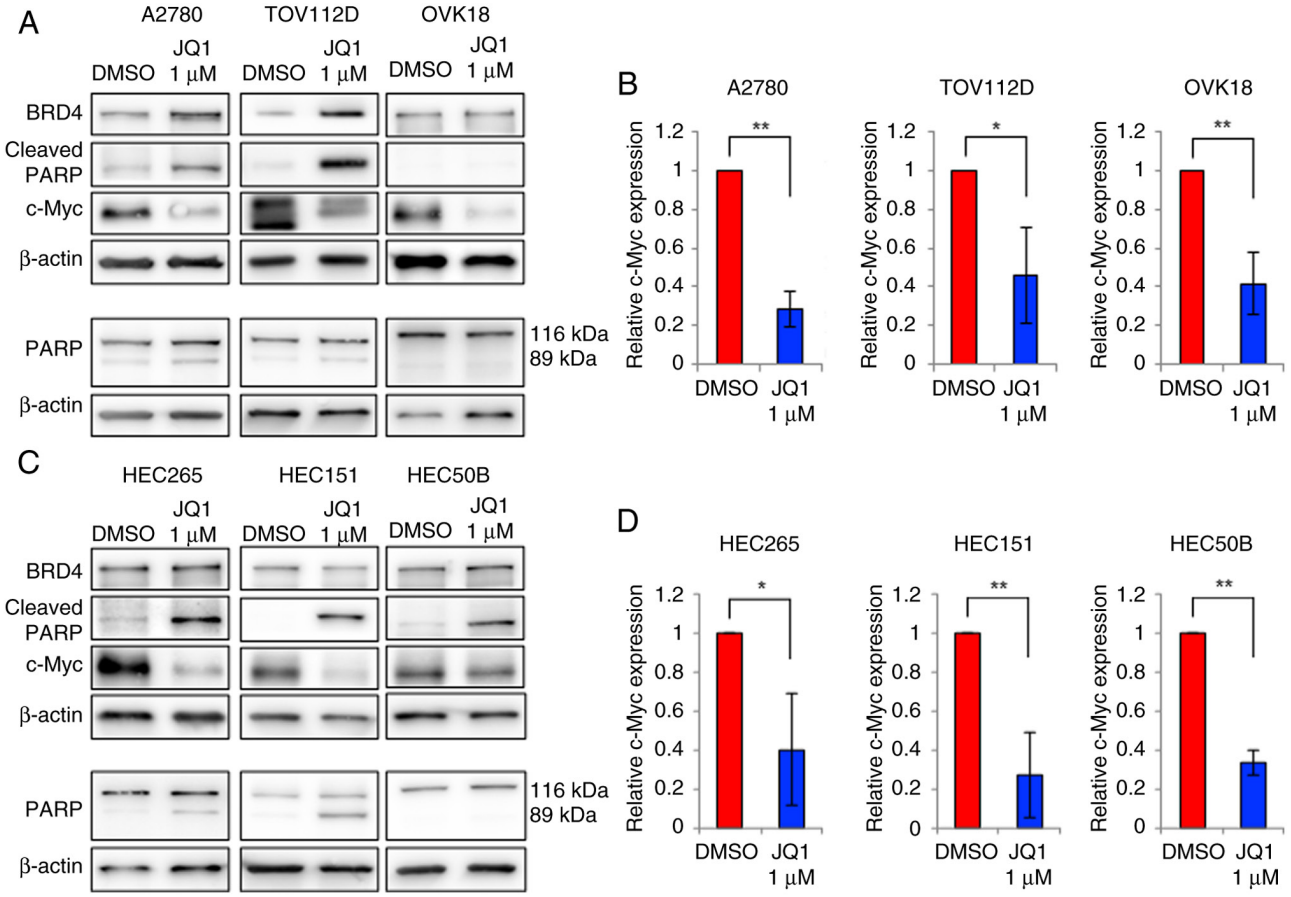


Figure 4. Western blotting analysis of the protein expression levels after JQ1 treatment. (A) BRD4, c-Myc, cleaved PARP, PARP and β-actin protein expression levels, and (B) relative c-Myc protein expression level in A2780, TOV112D and OVK18 cells treated with 0.1% DMSO or 1 μM JQ1 for 72 h. (C) BRD4, c-Myc, cleaved PARP, PARP and β-actin protein expression levels, and (D) relative c-Myc protein expression level in HEC265, HEC151 and HEC50B cells treated with 0.1% DMSO or 1 μM JQ1 for 72 h. The expression level of c-Myc decreased and that of cleaved PARP increased with the JQ1 treatment in all cell lines. *P<0.05; **P<0.01. BRD4, bromodomain 4; PARP, poly ADP ribose polymerase.

higher risk of developing ovarian endometrioid and clear cell carcinoma than those without endometriosis (34). A popular hypothesis for endometriosis development is the retrograde menstruation theory, which implies that the endometrium expelled as menstrual blood retrogradely enters the abdominal cavity and implants itself in pelvic organs, such as the peritoneum and ovaries, thus causing endometriosis (35,36). Notably, OEC is often associated with EEC of the endometrium (namely, SEOC) (37). Our previous study demonstrated a higher SEOC rate in patients with endometriosis than in those without (34). Moreover, the molecular and pathological features of low-grade OEC and EEC are similar (8,37). For example, mutations in cancer-related genes, such as *AT-rich interaction domain 1A* (*ARID1A*), *tumor protein P53*, *phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α*, *PTEN*, *KRAS* and *DNA polymerase ε catalytic subunit A*, are similar in OEC and EEC (38). These results support the hypothesis that endometria with carcinogenic changes may backflow from the uterus to the abdominal cavity, causing endometriosis in the ovaries. Consequently, the ovaries may become a reservoir for the further development of endometrial carcinoma (34). Therefore, the present study assessed JQ1 for the treatment of OEC and EEC.

The results of the present study indicate that JQ1 has antitumor effects in almost all the endometrial carcinoma cell lines evaluated. Moreover, exposing OEC and EEC cell lines to JQ1 inhibited cell proliferation. Notably, a high IC_{50} value was obtained in OVK18 cells treated with JQ1. In the colony formation assay, JQ1 showed efficacy in all cell lines, suggesting that long-term administration may be effective in all OECs and EECs. Furthermore, JQ1 induced apoptosis and suppressed *c-Myc* expression in ovarian and endometrial cancers. Different concentrations of JQ1 were used in each experiment as the optimal concentration of the target to be confirmed varied in each experiment. Moreover, the present study also performed experiments using the lowest feasible concentrations of JQ1 to demonstrate its effects. There are previous reports using JQ1 in A2780 cells (39,40); however, there are no previous reports in other cell lines, to the best of our knowledge.

A total of 1 μM was used for the western blot analysis (Fig. 4) as 1 μM JQ1 markedly reduced cell viability across all cell lines in the inhibitor experiment (Fig. 1). Previous studies using A2780 cells reported decreased *c-Myc* expression with 0.3 μM (39) and 2.5 μM (40) JQ1. In the colony formation assay (Figs. 2 and 3), lower concentrations of JQ1 exhibited effects, likely due to prolonged JQ1 administration. Considering previous research indicating colony formation suppression even at 0.1 μM (41,42), the present study assessed concentrations of 0.1, 0.2 and 0.5 μM to evaluate outcomes

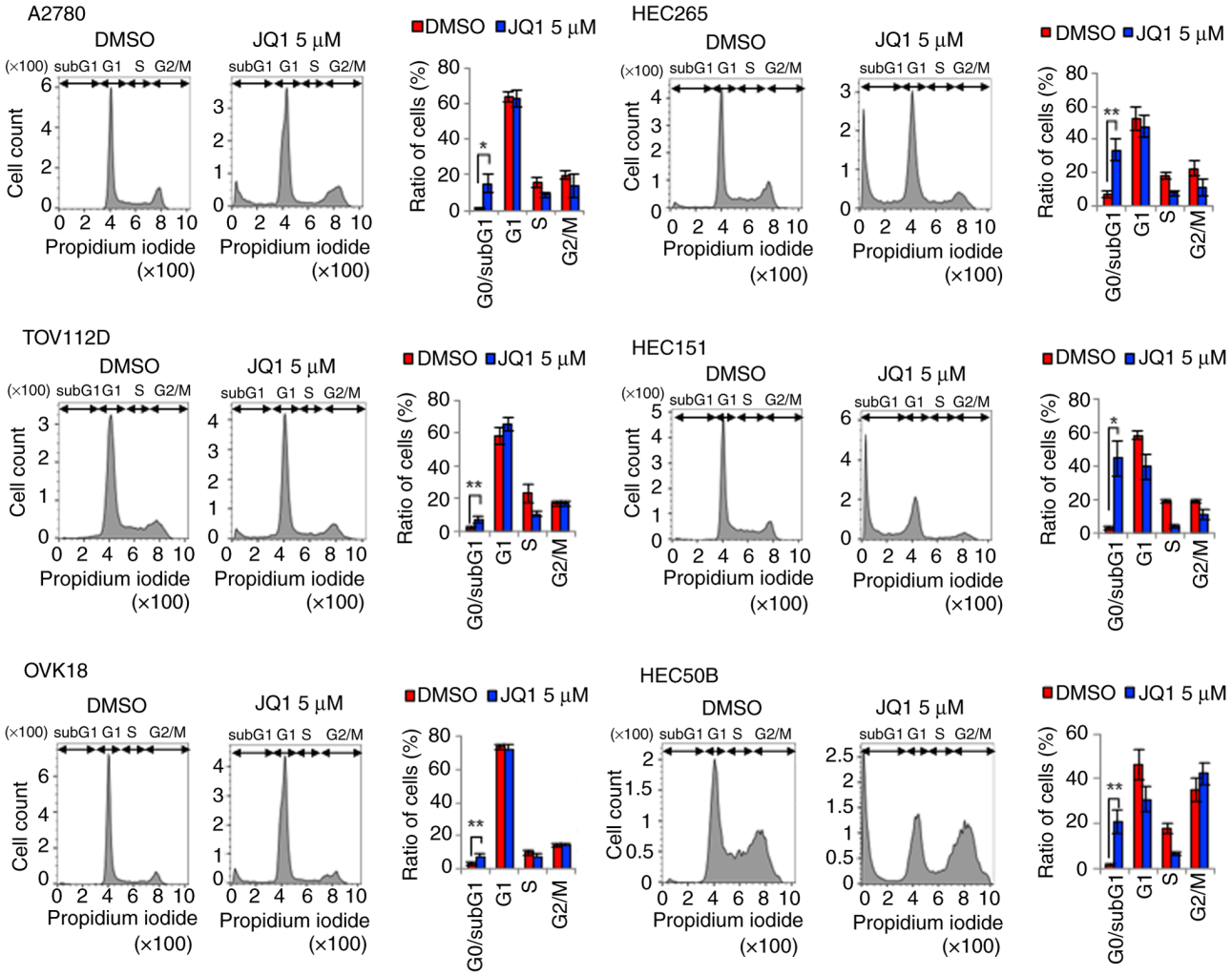


Figure 5. Cell cycle analysis of ovarian endometrioid carcinoma and endometrial endometrioid carcinoma cell lines treated with JQ1. Treatment with 5 μM JQ1 for 96 h significantly increased the cell population in the sub-G1 phase compared with 0.1% DMSO for all the cell lines. Flow cytometry and PI staining were performed to analyze the cell cycle phase. *P<0.05; **P<0.01, vs. 0.1% DMSO.

at lower concentrations. Furthermore, regarding the cell cycle assay (Fig. 5), a previous study (42) was referred to and pilot experiments at 2.5 and 5 μM concentrations were performed. As a significant increase in the sub-G1 phase was demonstrated at 5 μM across all cell lines, this concentration was selected for subsequent experiments.

Although JQ1 was initially designed as a selective inhibitor of BRDs, aiming to displace BRDs from chromatin and disrupt their role in regulating gene transcription (43), it is difficult to demonstrate that JQ1 inhibits BET function as there are no definite biomarkers that indicate that JQ1 inhibits BET protein. However, as JQ1 selectively binds to BET proteins to regulate transcription (43), and it is known to suppress *c-Myc* transcription (23,25), it can indirectly be inferred that when treatment with JQ1 leads to reduced c-Myc levels, it acts through BET proteins. Many studies have reported that JQ1 inhibits c-Myc and induces apoptosis (17,23,41,44). The findings of the present study were similar, suggesting that JQ1 inhibits BET protein and leads apoptosis via the inhibition of c-Myc.

Previous studies have reported the antitumor effects of BET inhibitors in ovarian and endometrial cancers. For example, Karakashev *et al* (30) reported that BET inhibitors enhance the sensitivity to PARP inhibitors in homologous recombination-proficient ovarian cancer. Liu *et al* (31) reported that i-BET151 (a BET inhibitor), when administered to ovarian cancer cell lines, promoted CD8-positive T cell infiltration and exhibited antitumor effects. Furthermore, i-BET151 was reported to demonstrate a synergistic effect with cisplatin by reducing survivin and B-cell lymphoma 2 levels in ovarian cancer cell line (32). Notably, the BET inhibitors GS-5829 and GS626510 have demonstrated antitumor effects against serous carcinoma cell lines, which belong to a special histological type of endometrial cancer (45). BET proteins regulate c-Myc levels, which are dysregulated in several cancers (46). c-Myc is known to be a critical transcription factor that regulates cell proliferation, differentiation and apoptosis (47,48). Previous studies have reported that suppression of c-Myc induces apoptosis in cancer (17,23,47,48). BET inhibitors, such as JQ1, have also been reported to suppress c-Myc expression in several cancers (including ovarian cancer) and exhibit antitumor effects (44,49). Qiu *et al* (41) reported that treating PTEN-positive uterine carcinoma with JQ1 inhibited cell growth and decreased c-Myc expression, which is consistent with the results of the present study.

Furthermore, a total of >50% of OECs have *ARID1A* mutations (3). Berns *et al* (40) reported that BET inhibitors were associated with synthetic lethality in *ARID1A* mutant ovarian cancer cells by reducing the available levels of SWI/SNF members, such as *ARID1B*. However, the present study revealed no differences in the antitumor effects of BET inhibitors on the *ARID1A* status of EEC and OEC cell lines.

The present study has certain limitations: i) As experiments on *c-Myc* overexpression were not performed, the mechanism of action of JQ1 was not elucidated. Future studies should perform additional experiments using BRD4 siRNA and *c-Myc* overexpression; ii) as the inhibition of c-Myc is known to induce apoptosis, the findings of the present study suggest that JQ1 may induce apoptosis via inhibition of c-Myc, but further studies are needed to prove this; and iii) analyses using TCGA database were performed, but not experiments using clinical samples. Therefore, future studies should assess the expression of BRD4 and its association with prognosis using clinical samples.

In conclusion, several reports on the antitumor effects of BET inhibitors in endometrial carcinoma have been published. Considering the findings of the present study, the BET inhibitor JQ1 may be effective in SEOC.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ST and KS conceived and designed the study. ST, KS, RHac, ES, NT, YT and FI designed the experiments. Experiments were performed by ST and YJ. YJ cooperated with the additional experiment. ST and KS confirm the authenticity of all the raw data. ST, KS, HH, TF, AT, YM, TI, MM, KA, MK, SK, RHam, OWH, KO, YH and YO contributed to the analysis and interpretation of the results. KS, KA, MK, SK, RHam, OWH, KO, YH and YO reviewed and revised the manuscript for important intellectual content. Technical and material support was provided by RH, ES, NT, YT, FI, TF and AT. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

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